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Acaricidal and repellent efficacy of *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* essential oils and their mixtures against *Rhipicephalus microplus* (Acari: Ixodidae) cattle ticks

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Abstract

Cattle tick (*Rhipicephalus microplus*) infestations cause significant losses in livestock. The study's goal was to demonstrate the efficacy of essential oils (EOs) derived from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* in killing livestock ticks, preventing them from reproducing by reducing oviposition and hatching. EOs are potential natural agents that have the ability to eliminate or deter ticks. They also provide an alternative to synthetic insecticides and can help prevent the spread of tick-borne diseases by reducing the risk of tick bites. The larval pack test demonstrated that *Azadirachta indica* (LC_{50} = 38.86 mg ml^{-1}), *Melaleuca alternifolia,* (LC_{50} = 26.76), and *Pogostemon cablin* (LC_{50} = 31.49) EOs killed all the ticks at a concentration of 20% (mg ml^{-1}) for 24 h post-treatment. Mixtures of EOs showed a high efficiency in killing ticks (100% mortality) at the fifth day of treatment at 5% (v/v) concentration. The treatment with EOs at 20% significantly reduced the oviposition (from 95.93 to 100.0%) of engorged female cattle ticks. We concluded that the pure and mixed EOs of *Melaleuca alternifolia* and *Pogostemon cablin* are very effective at killing ticks and stopping *R. microplus* reproduction.

Keywords Ticks · Acaricidal · Repellent · Essential oils · Control

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Introduction

Ticks are ectoparasites that feed on blood and belong to the order Ixodida, which consists of three families: Ixodidae, Argasidae, and Nuttalliellidae (Barker and Murrell 2004). Ticks are important next to mosquitoes as carriers of many infectious agents, such as viruses, spirochetes, bacteria, rickettsia, protozoa, and filarial nematodes (Batool et al. 2019; Mahmood et al. 2022). These pathogens can cause death in both people and animals. Tick-borne diseases (TBDs) are a significant obstacle to livestock development in many countries, especially in tropical and subtropical locations (Abd Elmohsen et al. 2019; Ali et al. 2020; Selim et al. 2020). They are a significant group of disease vectors, primarily affecting the cattle and camel industry (Selim et al. 2019; Aslam et al. 2023). Similarly, they are highly destructive, resulting in significant financial damage. Estimates place the losses incurred from frequent antiparasitic treatments and infestation impacts, such as blood loss, reduced weight gain and milk supply, and skin damage at the site of attachment, in the billions of US dollars (Alota et al. 2021).

Arsenicals, chlorinated hydrocarbons, carbamates, macrocyclic lactones, organophosphates, formamidines, pyrethroids, fluazuron, and fipronil are common tick insecticides. Farmers typically apply these substances by sprinkling, pouring, or injecting them into animals, which results in significant expenses (Coêlho et al. 2013; Nogueira et al. 2014).

It is too bad that the wrong, overuse, and inappropriate application of chemical acaricides has caused tick populations to become resistant (Castro et al. 2018; Alota et al. 2021; Luns et al. 2021). Nevertheless, in addition to their expensive nature, these acaricides also pose a potential risk by contaminating ruminant milk and meat (George et al. 2014; Quadros et al. 2020).

Consequently, they may have adverse effects on human health (Ellse and Wall 2014) and contribute to the contamination of the environment with harmful residues for both humans and animals (Castro et al. 2018; Quadros et al. 2020). The global market has restricted the use of certain acaricides, such as organochlorines, organophosphates, and pyrethroids, due to the growing interest in organic farming practices (Ellse and Wall 2014). Therefore, it is imperative to discover novel agents and/or effective alternative techniques to control them (Pazinato et al. 2014; Teixeira Pinto et al. 2018). As a result, we employ a variety of existing methods to manage tick populations that are resistant to treatment, such as biological control using pathogens or predators, pheromone-assisted control, herbal pour-on or dip preparations containing green manufactured nanoparticles, vaccination, and the development of acaricide resistance mitigation programs based on integrated pest management control (Pazinato et al. 2014; Teixeira Pinto et al. 2018).

Among these alternative methods, compounds derived from plants, especially EOs, show promise as acaricide sources (Pazinato et al. 2014; Abbas et al. 2018; Baz et al. 2024). The variety of chemicals in EOs and their interactions determine their acaricidal activity (Shezryna et al. 2020). Furthermore, due to their minimal toxicity and ability to dissolve in water (Shezryna et al. 2020), these substances can aid in the creation of milk and animal flesh devoid of hazardous chemicals that pose a threat to humans, animals, and the environment (de Oliveira Cruz et al. 2013). This paper provides a comprehensive analysis of tick control approaches, tick resistance, the use of EOs for tick control, and the specific processes by which they work.

Therefore, scientists extensively research EOs, derived from plants as secondary chemicals, for their insecticide, growth-regulating, repellant, or dissuasive characteristics (Baz et al. 2021; Hegazy et al. 2022; Al-Hoshani et al. 2023b) The variability in the chemical composition of EOs and the interrelationship between their components have a significant impact on acaricidal action (Camilo et al. 2017). The effectiveness of EOs lies in their biologically active components that kill the ticks, insects, and other microbes (Gonzaga et al. 2023). One of the effective oils is *Melaleuca alternifolia* oil, which contains several active ingredients such as γ-terpinene, 1,8-cineole, terpinen-4-ol, terpinene, p-cymene, and terpinolene (Borotová et al. 2022).

One way to make acaricide-based products is to combine different EOs, synthetic compounds with EOs (Ratajac et al. 2024), or synthetic compounds with EOs. This technique seeks to facilitate potential synergies, increase pest mortality rate, and reduce the quantity of each active ingredient required leading to rise the acaricides efficeincy (Jyoti and Maddheshiya 2023). Furthermore, it has the potential to decrease manufacturing expenses and mitigate the likelihood of environmental pollution (Jyoti and Maddheshiya 2023; Vale et al. 2023). We conducted a study to explore the effectiveness and eco-friendly nature of tick control compounds. In particular, we looked at how well EOs from lemongrass, geranium, savory thyme, and white thyme killed ticks. In addition, we examined the interaction (such as synergistic or additive) of their combined mixtures on the tick species.

Materials and methods

Essential oils and chemicals

Azadirachta indica (Meliaceae), *Boswellia serrata* (Burseraceae), *Melaleuca alternifolia* (Myrtaceae) and *Pogostemon cablin* (Lamiaceae), EOs were bought from Nefertari Company so that they could be used to make 100% pure EOs and therapeutic skin care products (Fig. 1). For

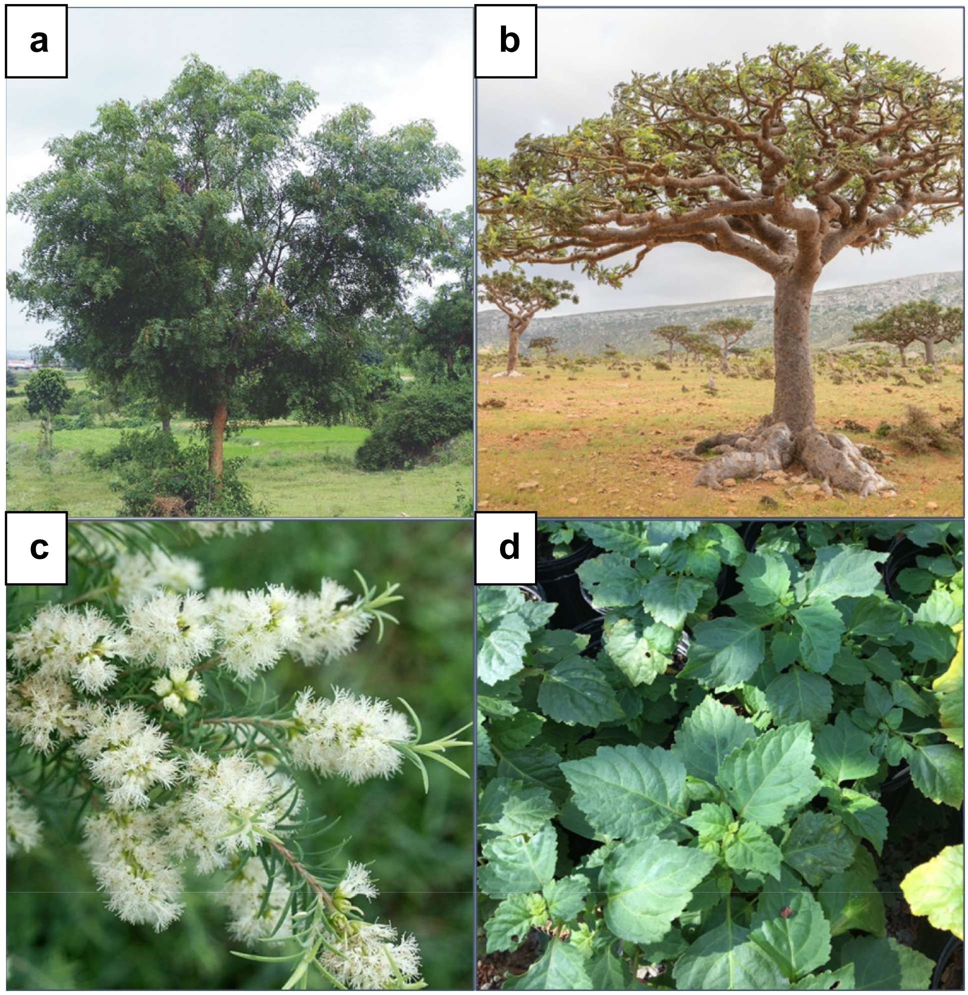

Fig. 1 Essential oils were derived from *Azadirachta indica* (**a**), *Boswellia serrata* (**b**), *Melaleuca alternifolia* (**c**) and *Pogostemon cablin* (**d**). (https://upload.wikimedia.org)

each oil we prepared 0.2 ml of Tween 80 as an emulsifier. Cypermethrin, with a purity of 89%, was utilized after dissolving in acetone according to the recommended dose.

Tick collection

A survey was conducted to collect ticks from livestock farms, including government institutions such as the farms of the College of Agriculture, the College of Veterinary Medicine, and the farms of local farmers. We manually collected approximately 100–300 larvae and 5–10 fully fed females of *Rhipicephalus microplus* (Canestrini, 1887) ticks as samples from each farm during 2024. ticks were stored in safe, well-ventilated glass containers with a small amount of green grass. The samples were stored at low temperatures and transported to the Medical Entomology Laboratory at the Faculty of Science, Benha University for laboratory experiments.

Larval packet test

The larval packet test (LPT) was conducted using the procedure outlined by the Food and Agriculture Organization of the United Nations (FAO 1984). A total of around one hundred larvae, aged between 10 and 12 days, were positioned between two filter sheets (2 × 2 cm) that were saturated with the corresponding EO (10, 25, 50, 100, and 200 mg ml^{-1}) and concentration, creating a sandwich-like arrangement. Each"sandwich"was placed inside a filter paper envelope and thereafter sealed, labeled, and kept in an incubator at a temperature of 27 °C with a relative humidity of at least 80% for a duration of 24 h (Leite 1988). After this period, we tallied the larvae, both living and deceased. Ticks that exhibited no signs of movement were deemed deceased. The experiment was conducted three times for each treatment. The mortality rate was determined by calculating the average of three replicates.

$$\text{Corrected mortality } (\%) = [(\% \text{ test mortality} - \% \text{ control mortality})/(100 - \% \text{ control mortality})] * 100$$

Effect of essential oil mixtures on the larvae of cattle ticks

A larval immersion technique was used to test the effect of the EO mixtures against larvae of cattle ticks, *R. microplus*, and to evaluate how well the mixture worked to kill larval ticks. The EO solutions were made by mixing 5 ml (50 mg/ml) of one EO with 94 ml of distilled water and 1 ml of Tween-80 to create a total of 100 ml, and this method was used in the larval packet test (LPT). For the EO mixture, 2.5 ml of each chosen oil was combined to make 5 ml of total oil, which was then mixed with 94 ml of distilled water and 1 ml of Tween-80 to reach a final volume of 100 ml. The Ultrasonic Homogenizer (Q500 sonicator) was used to blend the EOs together for 0.5 min. The oil mixing experiment was conducted only at a 5% concentration of EOs.

Adult immersion test

The adult immersion test (AIT) of fully engorged *R. microplus* (Canestrini 1887) females was performed following the procedure outlined by Drummond et al. (1973). Females who were swollen with blood were chosen based on their ability to move, their physical condition, and their size (≥4.5 mm). After being weighed, the females were divided into groups of twelve specimens each, based on their similar weights. The weight of fully fed females varied from 170 to 210 mg (Bennett 1974). Every cohort of female subjects was submerged in their respective treatment solution (10, 25, 50, 100, and 200 mg ml^{-1}) for a duration of 3 min, thereafter dried using paper towels, and then placed in a chamber with a temperature of 27 °C and a relative humidity of at least 80% for three weeks. The experiment was conducted using three replicates for each treatment.

Egg hatchability

Hatchability was determined by calculating the mean number of eggs and larvae. The egg production index (EPI), oviposition reduction (OR), reproductive efficiency (RE), and product effectiveness (PE) were computed using the following formulas: The EPI (Egg Production Index) is calculated by dividing the weight of eggs by the weight of engorged females and multiplying the result by 100 (Bennett 1974). The OR (Oviposition Rate) is determined by subtracting the treated EPI from the control EPI, dividing the result by the control EPI, and multiplying by 100 (Roulston et al. 1968). The RE (Reproductive Efficiency) is obtained by multiplying the weight of the egg mass by the percentage of eclosion and dividing the result by the weight of the mass of females, then multiplying by 20,000. Finally, the PE (Percentage Efficiency) is calculated by subtracting the treated RE from the control RE, dividing the result by the control RE, and multiplying by 100 (Drummond et al. 1973).

Bioassays

EOs were tested for their larvicidal efficacy according to the protocols by (WHO 2005) against *R. microplus*. The cattle ticks were treated with the following concentrations: 10, 25, 50, 100, and 200 mg ml^{-1} through the envelope technique (Zahir et al. 2010), a filter paper envelope, Whatman filter paper No. 1, 125 mm diameter, and after treatment, transport to a petri dish (7.5 × 7.5 cm). Three replicates (each containing ten tick larvae) were used for each concentration. The control envelopes were impregnated with distilled water. The opening of the envelopes was folded and secured with a metallic clip, along with identification marks like tested solution and concentration. *R. microplus* mortalities were recorded after 1, 3, 5 and 7 days post-treatment (PT) at 28 ± 2 °C and a relative humidity of 80%.

Phytochemical GC–MS analysis

Analyses of the selected EOs were performed using GC–MS. Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary columns (0.1 mm, 0.251 mm, and 30 m thick), were utilized for the GC–MS, which was employed for the biochemical analyses. It was achieved using an electronic ionizer with 70 eV of ionization energy. As a carrier gas, helium was used (flow rate: 1 ml/min). The MS transmission line and injector were both set to 280 °C. The oven was preheated to 50 °C, then increased to 150 °C at a rate of 7 °C per minute, 270 °C at a rate of 5 °C per minute, and finally 310 °C at a rate of 3.5 °C per minute. A relative peak area was employed to explore the quantification of all components discovered. The chemicals were at least partially identified by comparing the retention times and mass spectra of the chemicals to those of NIST and Willy Library data from the GC–MS instrument. Identification was done using the aggregate spectrum of user-generated reference libraries. To evaluate peak homogeneity, single-ion chromatographic reconstructions were performed. To verify GC retention periods, co-chromatographic analysis of reference substances was used whenever practical (Mostafa et al. 2024).

Data analysis

The biological data were subjected to one-way analysis of variance (ANOVA), Duncan's multiple range tests, and the lethal concentration of the EO for 50% of the population (LC_{50}) of larvae and engorged females was calculated through Probit analysis using the computer program PASW Statistics 2009 (SPSS version 22).

Results

Acaricidal activity of essential oils

The study showed that the EOs from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* were a successful method to get rid of the *R. microplus* cattle tick. At a concentration of 20% (mg ml^{-1}), these oils caused 100% mortality of the ticks after 24 h post-treatment, except *B. serrata* (96.67%) oil (Table 1). The LC_{50} values for *A. indica, B. serrata, M. alternifolia,* and *P. cablin* were 38.86, 47.13, 26.76, and 31.49 mg ml^{-1}, respectively (Table 2). At a concentration of 10%, *A. indica, B. serrata, M. alternifolia,* and *P. cablin* EOs were able to get rid of the cattle ticks with mortality rates 83.33%, 73.33%, 90.0%, and 83.33%, respectively.

Effect of the essential oil mixtures against larvae of cattle ticks *Rhipicephalus microplus*

Table 3 displays the effect of mixtures of AB (*Azadirachta* and *Boswellia*), AM (*Azadirachta* and *Melaleuca*), AP (*Azadirachta* and *Pogostemon*), BM (*Boswellia* and *Melaleuca*), BP (*Boswellia* and *Pogostemon*), and MP (*Melaleuca* and *Pogostemon*) EOs on *R. microplus* cattle ticks within

Table 1 Efficacy of essential oils from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* on larvae of cattle ticks *Rhipicephalus microplus*

Conc. (mg ml^{-1})	Mean larval mortality ± SD			
	Azadirachta indica	*Boswellia serrata*	*Melaleuca alternifolia*	*Pogostemon cablin*
0	0.00 ± 0.0^{fA}	0.00 ± 0.0^{fA}	3.33 ± 3.33^{fA}	0.00 ± 0.0^{fA}
10	13.33 ± 3.33^{eB}	13.33 ± 3.33^{eB}	20.00 ± 5.77^{eA}	16.67 ± 3.33^{eAB}
25	33.33 ± 3.33^{dC}	26.67 ± 3.33^{dD}	46.67 ± 3.33^{dA}	40.00 ± 0.00^{dB}
50	53.33 ± 3.33^{cB}	46.67 ± 3.33^{cC}	66.67 ± 3.33^{cA}	63.33 ± 3.33^{cA}
100	83.33 ± 3.33^{bC}	73.33 ± 3.33^{bC}	90.00 ± 0.00^{bA}	83.33 ± 3.33^{bB}
200	100.00 ± 0.00^{aA}	96.67 ± 3.33^{aB}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}
Positive control	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}

a, b & c: There is no significant difference ($p > 0.05$) between any two means for each plant, within the same column that have the same superscript letter; A, B & C: There is no significant difference ($p > 0.05$) between any two means, within the same row that have the same superscript letter. Positive control: Cypermethrin (89%)

Table 2 Lethal concentration (LC) of the essential oils from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* against *Rhipicephalus microplus* larvae

Plant extract	LC_{50}	LC_{90}	LC_{95}	R^2	Slope ± SE	Chi (sig.)
Azadirachta indica	38.86 (34.15–46.22)	176.70 (140.61–237.72)	267.60 (203.13–385.56)	0.978	2.015 ± 0.162	5.838 (0.119)
Boswellia serrata	47.13 (41.20–56.12)	232.10 (179.01–327.55)	362.55 (264.91–553.47)	0.991	1.876 ± 0.156	3.013 (0.389)
Melaleuca alternifolia	26.76 (22.94–30.78)	100.17 (82.69–127.85)	145.62 (115.56–196.92)	0.959	2.236 ± 0.178	5.012 (0.170)
Pogostemon cablin	31.49 (27.13–36.15)	120.28 (98.365–154.82)	175.86 (13.54–138.54)	0.944	2.202 ± 0.172	6.252 (0.100)

Table 3 Efficacy of blend essential oils from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* at 5% concentrations on mortality of larvae of the cattle tick *Rhipicephalus microplus*

Treatment	Mean larval mortality ± SD (%)			
	Day 1	Day 3	Day 5	Day 7
Control	0.00 ± 0.0^{eA}	0.00 ± 0.0^{eA}	3.33 ± 3.33^{bA}	3.33 ± 3.33^{bA}
Azadirachta + Boswellia (AB)	60.00 ± 5.77^{dD}	80.00 ± 5.77^{dB}	96.67 ± 3.33^{aA}	100 ± 0.00^{aA}
Azadirachta + Melaleuca (AM)	80.00 ± 0.00^{aC}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Azadirachta + Pogostemon (AP)	73.33 ± 6.67^{cD}	90.00 ± 0.00^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Boswellia + Melaleuca (BM)	70.00 ± 5.77^{cD}	90.00 ± 0.00^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Boswellia + Pogostemon (BP)	63.33 ± 3.33^{bdD}	80.00 ± 5.77^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Melaleuca + Pogostemon (MP)	83.33 ± 3.33^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

a, b & c: There is no significant difference ($p > 0.05$) between any two means, within the same column that have the same superscript letter; A, B & C: There is no significant difference ($p > 0.05$) between any two means, within the same row that have the same superscript letter

FIFTH and seven days after treatment except AB (96.67%). At day three, AM (*Azadirachta* and *Melaleuca*) and MP (*Melaleuca* and *Pogostemon*) EOs caused 100% mortality of the ticks (Fig. 2).

Effect of essential oils on engorged female's cattle ticks *Rhipicephalus microplus*

In engorged females, the four EOs, at 200 mg ml^{-1}, significantly reduced oviposition (ranging from 95.93 to 100.0%) as shown in Fig. 3, egg production index (0.0 to 3.59%) compared to 88.19 in control femal groupes (Fig. 3c), and hatchability (0.0 to 16.0%), exhibiting an average efficiency that reached 100%, but there was no significant difference among them at this concentration ($p > 0.05$). This effect on engorged females was similar to that induced by the positive control (Table 4).

GC–MSGC–MS identification of the chemical composition of essential oils

Phytochemical analysis was carried out using a GC–MS chromatogram for *Azadirachta indica*, *Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* (Tables 5, 6, 7, and 8). Tributyl acetylcitrate (37.39%), phytol (11.74%), and α-pinene (10.195) were found to be the main chemicals in *Azadirachta indica* oil (Table 5).

Methyl 9-cis,11-trans-octadecadienoate (36.55%), 9-Octadecenoic acid, methyl ester, (E)- (27.41%), and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (20.52%) were more abundant in *Boswellia serrata* oil (Table 6). 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (27.26%), Caryophyllene (15.16%), and

Fig. 3 Effect of essential oils: C - control, Ai - *Azadirachta indica,* Bs - *Boswellia serrata,* Ma - *Melaleuca alternifolia,* and Pc - *Pogostemon cablin* on engorged female cattle ticks *Rhipicephalus microplus*

ç-Terpinene (11.07%) were more common in *Melaleuca alternifolia* oil (Table 7). Greater abundance was found for Patchouli alcohol (32.12%), α-Guaiene (20.21%), and Caryophyllene (11.51%) in *Pogostemon cablin* oil; 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (21.58%), Caryophyllene

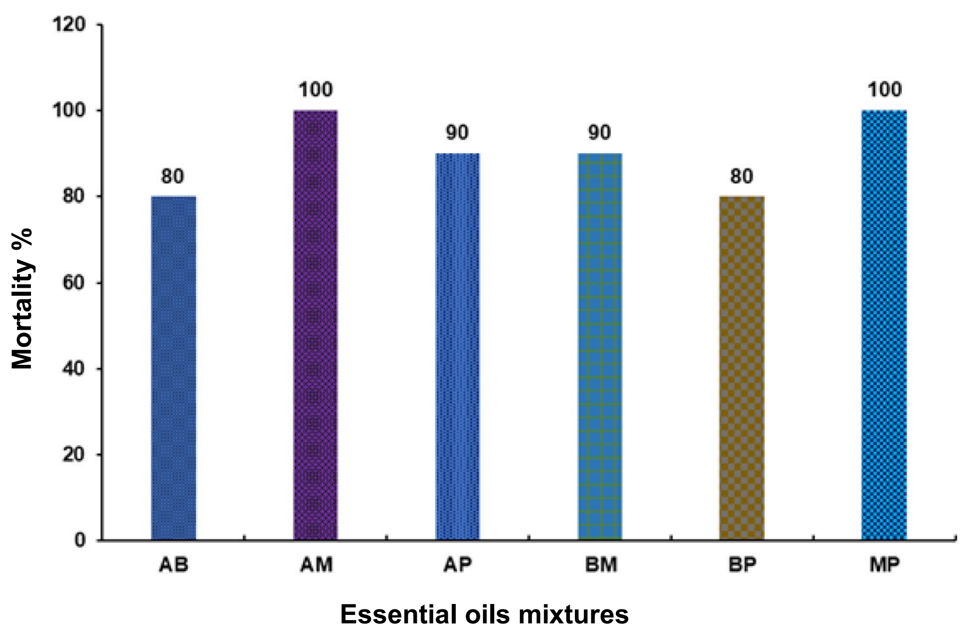

Fig. 2 The mean larval mortalities induced by the effects of essential oils of *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* against larvae of cattle ticks *Rhipicephalus microplus*, three days post-exposure. AB (*Azadirachta* and *Boswellia*), AM (*Azadirachta* and *Melaleuca*), AP (*Azadirachta* and *Pogostemon*), BM (*Boswellia* and *Melaleuca*), BP (*Boswellia* and *Pogostemon*), MP (*Melaleuca* and *Pogostemon*)

Table 4 Egg production index (EPI), reduction in oviposition (RO), eggs hatched, and efficiency (EP) of essential oils from *Azadirachta indica, Boswellia serrata, Melaleuca alternifolia,* and *Pogostemon cablin* on engorged females of *Rhipicephalus microplus*

Parameter	Conc mg ml^{-1}	Plant extract			
		A. indica	*B. serrata*	*M. alternifolia*	*P. cablin*
Egg production index (EPI)	0	88.19 ± 5.01^{aA}	88.19 ± 5.01^{aA}	88.19 ± 5.01^{aA}	88.19 ± 5.01^{a}A
	10	73.21 ± 2.74^{bA}	67.23 ± 4.27^{bB}	33.11 ± 3.19^{bD}	43.69 ± 5.95^{bC}
	25	47.84 ± 3.61^{cA}	39.16 ± 5.59^{cB}	16.01 ± 1.04^{cC}	17.16 ± 1.32^{cC}
	50	22.19 ± 5.40^{dB}	34.92 ± 3.57^{dA}	8.07 ± 5.57^{dC}	9.80 ± 4.23^{dC}
	100	9.20 ± 4.69^{eAB}	11.80 ± 3.08^{eA}	0.0 ± 0.0^{eC}	4.94 ± 4.39^{deB}
	200	0.93 ± 0.46^{fA}	3.59 ± 2.93^{fA}	0.0 ± 0.0^{eA}	0.0 ± 0.0^{eA}
Reduction in oviposition (RO)	0	-0.01 ± 5.68^{fA}	-0.01 ± 5.68^{eA}	-0.01 ± 5.68^{eA}	-0.01 ± 5.68^{eA}
	10	16.99 ± 3.10^{eD}	23.77 ± 4.85^{dC}	62.46 ± 3.62^{dA}	50.46 ± 6.75^{dB}
	25	45.76 ± 4.09^{dC}	55.60 ± 6.34^{cB}	81.85 ± 1.18^{cA}	80.54 ± 1.49^{cA}
	50	74.84 ± 6.12^{cB}	60.40 ± 4.05^{cC}	90.85 ± 6.31^{bA}	88.89 ± 4.79^{bA}
	100	89.56 ± 5.32^{bBC}	86.61 ± 3.49^{bC}	100 ± 0.00^{aA}	94.40 ± 4.98^{abB}
	200	98.95 ± 0.52^{aA}	95.93 ± 3.32^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{a}A
Product effectiveness (PE)	0	-0.01 ± 6.44^{eA}	-0.01 ± 6.44^{eA}	-0.01 ± 6.44^{dA}	-0.01 ± 6.44^{dA}
	10	33.56 ± 10.61^{dC}	35.59 ± 12.11^{dC}	80.02 ± 1.65^{cA}	68.57 ± 4.52^{cB}
	25	60.85 ± 7.20^{cB}	61.89 ± 12.29^{cB}	91.07 ± 2.37^{bA}	87.61 ± 5.2^{bA}
	50	83.82 ± 7.50^{bB}	74.49 ± 7.21^{bC}	98.00 ± 1.08^{abA}	96.12 ± 1.9^{aA}
	100	97.94 ± 1.26^{aA}	94.92 ± 2.68^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	200	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Hatching (%)	0	95.70 ± 0.90^{aA}	95.70 ± 0.90^{aA}	95.70 ± 0.90^{aA}	95.70 ± 0.90^{aA}
	1	76.30 ± 11.04bA	80.30 ± 12.36^{bA}	51.47 ± 4.56^{bB}	60.77 ± 3.23^{bB}
	2.5	71.10 ± 16.02^{bA}	77.77 ± 18.98^{bA}	46.90 ± 12.42^{bcB}	57.77 ± 21.19^{bB}
	5	55.77 ± 12.12^{cA}	59.77 ± 10.76^{cA}	33.47 ± 14.27^{cB}	29.33 ± 5.81^{cB}
	10	16.00 ± 7.02^{dB}	33.33 ± 13.33^{dA}	0.0 ± 0.0^{dC}	0.0 ± 0.0^{dC}
	20	0.0 ± 0.0^{eA}	16.00 ± 7.02^{eA}	0.0 ± 0.0^{eA}	0.0 ± 0.0^{dA}
Product effectiveness (PE)	0	-0.01 ± 6.44^{eA}	-0.01 ± 6.44^{eA}	-0.01 ± 6.44^{dA}	-0.01 ± 6.44^{dA}
	10	33.56 ± 10.61^{dC}	35.59 ± 12.11^{dC}	80.02 ± 1.65^{cA}	68.57 ± 4.52^{cB}
	25	60.85 ± 7.20^{cB}	61.89 ± 12.29^{cB}	91.07 ± 2.37^{bA}	87.61 ± 5.2^{bA}
	50	83.82 ± 7.50^{bB}	74.49 ± 7.21^{bC}	98.00 ± 1.08^{abA}	96.12 ± 1.9^{aA}
	100	97.94 ± 1.26^{aA}	94.92 ± 2.68^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	200	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	P.O.*	98.00 ± 1.08^{abA}			

a, b & c: There is no significant difference ($p>0.05$) between any two means for each perameter, within the same column that have the same superscript letter; A, B & C: There is no significant difference ($p>0.05$) between any two means, within the same row that have the same superscript letter. * P.O.: Positive control

(14.66%), and 1-Phenylethanone (9.70%) in *Pogostemon cablin* oil (Table 8).

Discussion

Bioactive plant chemicals, pheromones, and microorganisms like bacteria, fungi, viruses, or protozoa are all natural sources of bioinsecticides. Based on where they come from, the four main types of bioinsecticides are phytochemicals, microbial pesticides, plant-incorporated protectants, and pheromones (Prabha et al. 2016; Chengala and Singh 2017). They have been used successfully in pest control (Prabha et al. 2016). They are superior to synthetic compounds because they are less toxic, target-specific, extremely effective in small doses, and biodegradable.

From the present data, it is clear that the EOs of *A. indica, B. serrata, M. alternifolia,* and *P. cablin* have pronounced activity against the cattle tick *R. microplus*, where 100% mortality was reached after 24 h of 20% (mg ml^{-1}) exposure, especially for *A. indica, M. alternifolia,* and *P. cablin* oils. Within seven days, we observed the strong effect of EO mixtures against the larvae of the cattle tick *R. microplus*. Among those mixtures was a mixture of *Azadirachta* and *Melaleuca*

Table 5 The major chemical constituents of *Azadirachta indica* oil

No	RT	Compound name	Area (%)	M. F	Classification
1	21.16	Neophytadiene	2.80	$C_{20}H_{38}$	fatty acid
2	24.31	1-Dodecanol, 3,7,11-trimethyl-	0.52	$C_{15}H_{32}O$	fatty alcohol
3	24.94	17-Octadecynoic acid	1.51	$C_{18}H_{32}O_2$	linoleic Acid
4	25.61	Pentadecanoic acid, 14-methyl-, methyl ester	1.42	$C_{17}H_{34}O_2$	fatty acid
5	26.24	α-pinene	**10.19**	$C_{10}H_{16}$	monoterpene
6	26.42	Hexadecanoic acid	5.55	$C_{16}H_{32}O_2$	fatty acid
7	28.78	9-Octadecenoic acid (z)-, methyl ester	3.01	$C_{19}H_{36}O_2$	fatty acid
8	29.09	Phytol	**11.74**	$C_{20}H_{40}O$	fatty acid
9	29.22	Terpinen-4-ol	5.50	C10H18O	monoterpene
10	29.58	Pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxy-propyl)-, hydrazide	4.97	$C_{12}H_{24}N_2O_3$	phenol
11	30.01	Oleic Acid	1.05	$C_{18}H_{34}O_2$	fatty acid
12	31.27	Tributyl acetylcitrate	**37.39**	$C_{20}H_{34}O_8$	fatty acid
13	40.91	Hexadecadienoic acid, methyl ester	1.83	$C_{17}H_{30}O_2$	esters
14	41.87	Linoleic acid, 2,3-bis-(O-TMS)-propyl ester	7.95	$C_{27}H_{54}O_4Si_2$	sesquiterpene
15	42.31	1-Heptatriacotanol	4.57	$C_{37}H_{76}O$	sesquiterpene

Table 6 The major chemical constituents of *Boswellia serrata* oil

No	RT	Compound name	Area (%)	M. F	Classification
1	24.77	α -Phellandrene, dimer	0.30	$C_{20}H_{32}$	sesquiterpene
2	25.12	α-pinene	3.12	$C_{10}H_{16}$	monoterpene
3	27.38	Hexadecanoic acid, methyl ester	8.16	$C_{17}H_{34}O_2$	fatty acid
4	29.31	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	**20.52**	$C_{19}H_{34}O_2$	methyl esters
5	30.56	p-cymene	2.11	$C_{10}H_{14}$	monoterpene
6	30.65	Methyl 9-cis,11-trans-octadecadienoate	**36.55**	$C_{19}H_{34}O_2$	fatty acid
7	30.83	9-Octadecenoic acid, methyl ester, (E)-	**27.41**	$C_{19}H_{36}O_2$	fatty acid
8	31.28	Methyl stearate	1.83	$C_{19}H_{38}O_2$	fatty acid

Table 7 The major chemical constituents of *Melaleuca alternifolia* oil

No	RT	Compound name	Area (%)	M. F	Classification
1	4.42	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	1.55	$C_{10}H_{16}$	monoterpene
2	4.54	α -Pinene	2.94	$C_{10}H_{16}$	monoterpene
3	5.92	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	0.55	$C_{10}H_{16}$	monoterpene
4	6.31	Benzene, 1-methyl-3-(1-methylethyl)-	7.57	$C_{10}H_{14}$	monoterpene
5	6.46	Eucalyptol	4.29	$C_{10}H_{18}O$	monoterpene
6	7.22	γ -Terpinene	**11.07**	$C_{10}H_{16}$	monoterpene
7	7.56	Methyl 9-cis,11-trans-octadecadienoate	5.22	$C_{19}H_{34}O_2$	fatty acid
8	7.91	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	2.22	$C_{10}H_{16}$	monoterpene
9	9.06	cis-p-2-menthen-1-ol	4.25	$C_{10}H_{18}O$	monoterpene
10	10.18	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	**27.26**	$C_{10}H_{18}O$	monoterpene
11	10.41	α-Terpineol	5.66	$C_{10}H_{18}O$	monoterpene
12	12.26	3-Hexyne-2,5-diol, 2,5-dimethyl-	1.86	$C_8H_{14}O_2$	phenol
13	14.83	α-Cubebene	1.22	$C_{15}H_{24}$	sesquiterpene
14	16.46	Caryophyllene	**15.16**	$C_{15}H_{24}$	sesquiterpene
15	17.76	Linoleic acid, 2,3-bis-(O-TMS)-propyl ester	2.24	$C_{15}H_{24}$	sesquiterpene
16	18.77	(E)-Calamenene	5.25	$C_{15}H_{22}$	sesquiterpene
17	29.03	Terpinyl butyrate	2.69	$C_{14}H_{24}O_2$	fatty acid

Table 8 The major chemical constituents of *Pogostemon cablin* oil

No	RT	Compound name	Area (%)	M. F	Classification
1	5.11	α-Pinene	2.46	$C_{10}H_{16}$	Monoterpene
2	12.26	Copaene	0.81	$C_{15}H_{24}$	sesquiterpene
3	16.15	α-Cedrane	3.80	$C_{15}H_{24}$	sesquiterpene
4	18.56	1-ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl)cyclohexane	1.45	$C_{15}H_{24}$	sesquiterpene
5	18.68	Caryophyllene	**11.51**	$C_{15}H_{24}$	sesquiterpene
6	20.23	α-Guaiene	**20.21**	$C_{15}H_{24}$	sesquiterpene
7	20.78	Humulene	0.40	$C_{15}H_{24}$	sesquiterpene
8	20.80	1-Phenylethanone	**9.70**	$C_9H_{11}NO_2$	phenol
9	22.55	α-Maaliene	3.62	$C_{15}H_{24}$	sesquiterpene
10	24.11	2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)−2-butenal	0.82	$C_{14}H_{22}O$	sesquiterpene
11	24.45	Caryophyllene oxide	5.56	$C_{15}H_{24}O$	sesquiterpene
12	26.76	(-)-Globulol	4.19	$C_{15}H_{26}O$	sesquiterpene
13	26.99	Patchouli alcohol	**32.12**	$C_{15}H_{26}O$	sesquiterpene
14	27.91	6-Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1 h-naphthalen-2-one	0.37	$C_{15}H_{22}O$	sesquiterpene
15	28.28	Ethanone, 1-(2,4,6-trihydroxyphenyl)-	2.98	$C_8H_8O_4$	phenol

(AM) and *Melaleuca* and *Pogostemon* (MP), which had a significant effect on killing cattle tick larvae from the third day until the seventh day. Many studies have shown the powerful toxicity of tea oil against adult and larval cattle and camel ticks, in addition to its strong effect against many other pests (Baz et al. 2024; Ratajac et al. 2024).

There has been extensive research in the last decade into the repellent and acaricidal effects of many EOs against ticks. A study investigated how tea tree oil (TTO) from *M. alternifolia* affected female *R. microplus* reproduction, finding that TTO in its pure (5 and 10%) and nanostructured (0.375 and 0.75%) forms stopped all reproduction (Pazinato et al. 2014). Yim et al. (2016) examined the repellent effects of *M. alternifolia* (tea tree) oil against cattle tick larvae (*Rhipicephalus australis* (Fuller, 1899)). At 48 h, 5% TTO provided 78% repellency, but lower concentrations repelled less than 60% of larvae. Alimi et al. (2024) investigate the acaricidal and repellent activities, as well as the putative mode of action of two EOs from *M. alternifolia* and *Chamaemelum nobile* (Roman chamomile) on *Hyalomma scupense* (Schulze, 1919). They showed that *M. alternifolia* achieved 100% repellency at a concentration of 1 mg ml^{-1}, whereas *C. nobile* showed 95.98% repellency activity at a concentration of 4 mg mL^{-1}.

The effects of *M. alternifolia* oil were tested (pure and in nanocapsules) in the control of *R. microplus* in dairy cattle. Treatment with TTO in nanocapsules interfered with *R. microplus* reproduction, leading to lower oviposition by female ticks and hatchability (34.5% of efficacy). On the other hand, TTO oil did not interfere with ticks reproduction, i.e., showed higher hatchability than the control group (Boito et al. 2016). Coulibaly et al. (2023) looked at how well different combinations of EOs from *Ocimum americanum*, *O. gratissimum*, and *Lippia multiflora* killed *R. microplus* larvae in a lab setting. The EO from *O. gratissimum* was the most effective, with LC_{50} values of 10.36 mg ml^{-1} and LC_{90} values of 15.51 mg ml^{-1}.

In one of the rare trials in the latter group, goats were protected from tick bites by using soap containing 0.03 µl g^{-1} of *Ageratum houstonianum* EO. However, it is unclear how much concentration the goat actually received based on the described approach. Goats receiving treatment saw a 95% decrease in biting ticks eight days later, compared to a 23% decrease in the soap-only control group (Pamo et al. 2005). It is unclear if this was due to mortality, irritation that caused detachment, or the soap or oil acting as a repellant to prevent further questing ticks from attaching.

Arthropod susceptibility also depends on the toxicity of the EO. Therefore, the quality of the oil has a significant impact on the rate of tick death, in addition to the frequent or multiple use of EOss from multiple plant sources, which gives good and clear results for exploring some oils that have a strong effect on eliminating ticks and many other pests (Yasmin et al. 2020; Štrbac et al. 2021). Other EOs from *Araucaria heterophylla, Eucalyptus camaldulensis, Pinus halepensis, Cyperus rotundus, Mentha arvensis,* and *Rosmarinus officinalis* had a clear effect on killing camel tick larvae of *Hyalomma dromedarii* (Koch, 1844) and *Culex pipiens* (Linnaeus, 1758) at a concentration of 10% (Baz et al. 2024). Some studies found that EOs have repellent effects, such as the EO of *Gynandropsis gynandra* (Lwande et al. 1999), two varieties of *Nepeta cataria* EO, and iridoid nepetalactone (Birkett et al. 2011), which deter unfed ticks from questing. A concentration of 0.1 µL of *G.*

gynandra oil was shown to repel 98.9% of nymphs of *Rhipicephalus appendiculatus* (Neumann, 1901) from questing; the deterring effects of the oil's major constituents were not as effective as those of the oil as a whole, suggesting a synergistic effect (Lwande et al. 1999). Through GC–MS analysis, two different strains of EO of catnip were shown to have marked differences in chemical composition that were associated with significant differences in effect: 50% repellency dosages (RD50) were 0.05 mg and 0.0012 mg, respectively (Birkett et al. 2011).

The susceptibility of adult *Rhipicephalus* spp. to EOs appears generally to be substantially lower than that of the larvae of this genus (Ribeiro et al. 2010). Researchers found that engorged adults of *Rhipacephalus* spp. immersed in 5% EO of geranium (*Pelargonium roseum*) (Pirali-Kheirabadi et al. 2009) and 0.8% EO of oregano (*Origanum bilgeri*) (Koc et al. 2013) had mortalities of 79.2% after 24 h and 83.3% after 48 h, respectively.

A significant decrease in the egg mass laid by female *Rhipicephalus* spp. was observed after immersion in the EOs of *Lippia triplinervis* (Lage et al. 2013), *Pelargonium roseum*, *Eucalyptus globulus* (Pirali-Kheirabadi et al. 2009), and *Hesperozygis ringens* (Ribeiro et al. 2010), suggesting that sublethal doses of EO may have an effect on tick fecundity. Adults exposed to EO may also affect the viability of their eggs. Immersion in the oil of *L. triplinervis* caused a significant decrease in tick egg hatching (Lage et al. 2013).

A phytochemical screening revealed the presence of terpenes, flavonoids, fatty acids, and phenols in tea tree oil and patchouli. Secondary metabolites like alkaloids, carbohydrates, flavonoids, saponins, tannins, and terpenoids are what give medicinal plants their pharmacological effects. These materials also have a strong effect on killing ticks and many insect pests. We conducted phytochemical analysis using a GC–MS chromatogram for *A. indica, B. serrata, M. alternifolia,* and *P. cablin*. *Pogostemon cablin* oil contained higher levels of Patchouli alcohol (32.12%), α-Guaiene (20.21%), and Caryophyllene (11.51%) compared to tea tree oil. In contrast, tea tree oil was characterized by higher concentrations of 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (27.26%), Caryophyllene (15.16%), γ-Terpinene (11.07%), and Benzene, 1-methyl-3-(1-methylethyl)- (7.57%).

In addition to their ability to control pests, research indicates that many medical treatments frequently use tea tree oil and patchouli. This may be because it can make aromatic phytochemical compounds that are toxic to pests (Yunus et al. 2022; Zhang and Piao 2023). According to the current results, *M. alternifolia,* and *P. cablin* EOs were the best at affecting pests and the most distinguished for containing secondary biological compounds, where the most prevalent classes of compounds in *M. alternifolia*, and *P. cablin* were sesquiterpenes and monoterpene being the main compounds. There is also a chance that these compounds have toxicity against microbes and other pests, as well as vector-borne (Zaman et al. 2012; Al-Hoshani et al. 2023a; Ündağ and Dönmez 2023). Researchers from all over the world have studied plants for their therapeutic benefits due to their antioxidant activity, commercial feasibility, and side effects (Xu et al. 2017; Baz et al. 2021).

Conclusions

We must keep animal healthy and safe from diseases spread by ticks, as livestock serves as an important source of food. Conventional insecticides are still the main way people around the world try to get rid of parasites and insects, but ticks and insects are becoming resistant to almost all types of insecticides. Due to the wide diversity and high potency of many plant-borne compounds, plants and EOs, as environmentally friendly insecticides, represent safe and suitable alternatives. Our results showed that EOs were effective in killing ticks, especially when mixed together, and this is due to their containing multiple secondary biological compounds. We found that tea tree oil and patchouli were the most effective EOs against cattle tick *R. microplus*.

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